COMPARISON OF CYTOKINE CONCENTRATIONS IN ULTRAFROZEN VS LYOPHILISED PLASMA FROM MDS PATIENTS BY LUMINEX

S. Muntión[1], M. García-Antunez[1], Marta Martín-Ayuso[2], Miguel G. Alvarez[2], Ana Hernández[2], Ana Gómez de la Torre[3], Concepción Rodríguez Serrano[3], Teresa Prieto[1], Raúl Azibeiro[1], Marta Fonseca[1], Carlos Puertas[1] and María Díez Campelo[1].

1. Hospital Universitario de Salamanca-IBSAL, Hematology, Salamanca, Spain, 2. 300K Solutions, Salamanca, Spain , 3. Centro de Investigación del Cáncer (CIC), Lab.12–Hematology, Salamanca, Spain

INTRODUCTION

Although in myelodysplastic syndromes (MDS) most studies focus on haematopoietic progenitors, the profile of cytokines present in plasma is poorly understood. In this regard, preserving the quality of this type of biological sample in crucial for its diagnostic applications.

Currently, the gold-standard for plasma storage is ultra-low temperature (ULT) freezing at -80ºC. However, this approach implies high maintenance costs, large spaces, constant energy supply and safety measures to minimized the risk of missing collections. One of the alternatives to ULT freezing is freeze-drying, a methodology for dehydrating biological samples that allows them to be kept at room temperature for long periods of time. In this context, 300K Solutions has developed a proprietary technology that provides a tool to stabilise dry samples, combining technology in precision lyophilisers, with sample protection reagents and vial formats that maintain traceability in laboratories.

AIM

To measure the profile of cytokines of patients diagnosed with MDS and compare their concentrations between the plasma stored RT vs freezing at -80ºC.

METHODS

Plasma samples were obtained from peripheral blood (PB) of 15 patients with MDS (LB-MDS, RS-MDS and HB-MDS). Then, two plasma aliquots from each patients were stored at-80ºC. After 2 months of freezing, a cryovial was thawed and centrifuged 3000g 20'. 500ul were kept in the refrigerator and another 500ul were lyophilised using the plasma and serum Stabilization Kit 24 of 300K Solutions. After the freeze-drying process, each plasma sample was rehydrated and together with its thawed and refrigerated, we analized using multiplex bead array assays (Luminex) to detect 22 cytokines involved in the pathogenesis of this disease comparing PB plasma vs PB lyophilised plasma (L-PB)

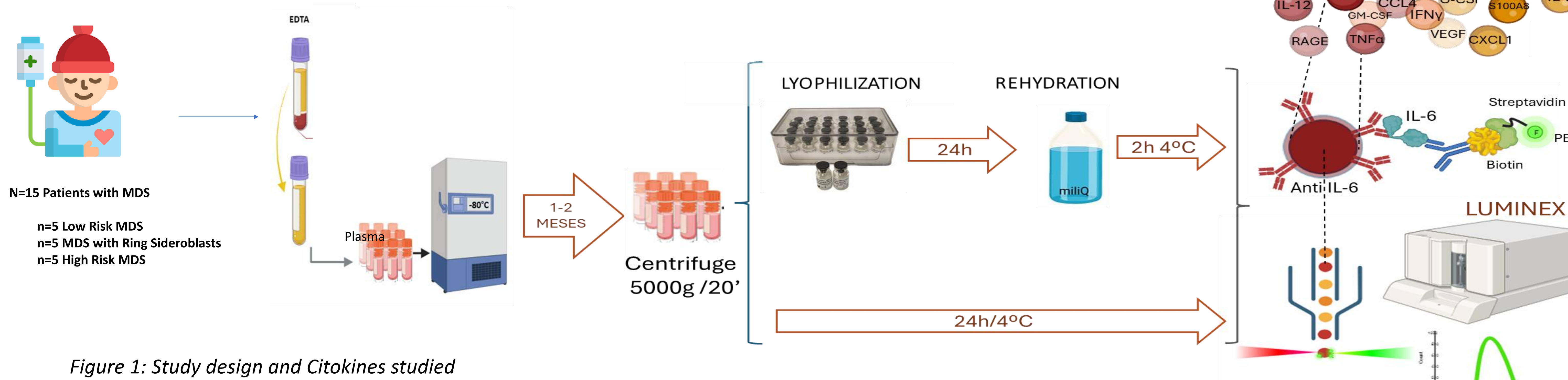

Figure 1: Study design and Citokines studied

RESULTS

Among the 22 cytokines studied, only 1 was affected by the lyophilization process (CXCL10), while 7 cytokines showed a variable pattern, with lyophilisation preserving them better in some cases and freezing in others (IL-6, IL-8, S100A8, VEGFa, FASL, CCL2, Ang) Figure 2. This variability may be due to different issues impacting the pre-analytical phases. The remaining 16 cytokines showed no significant differences between the two stabilization methods.

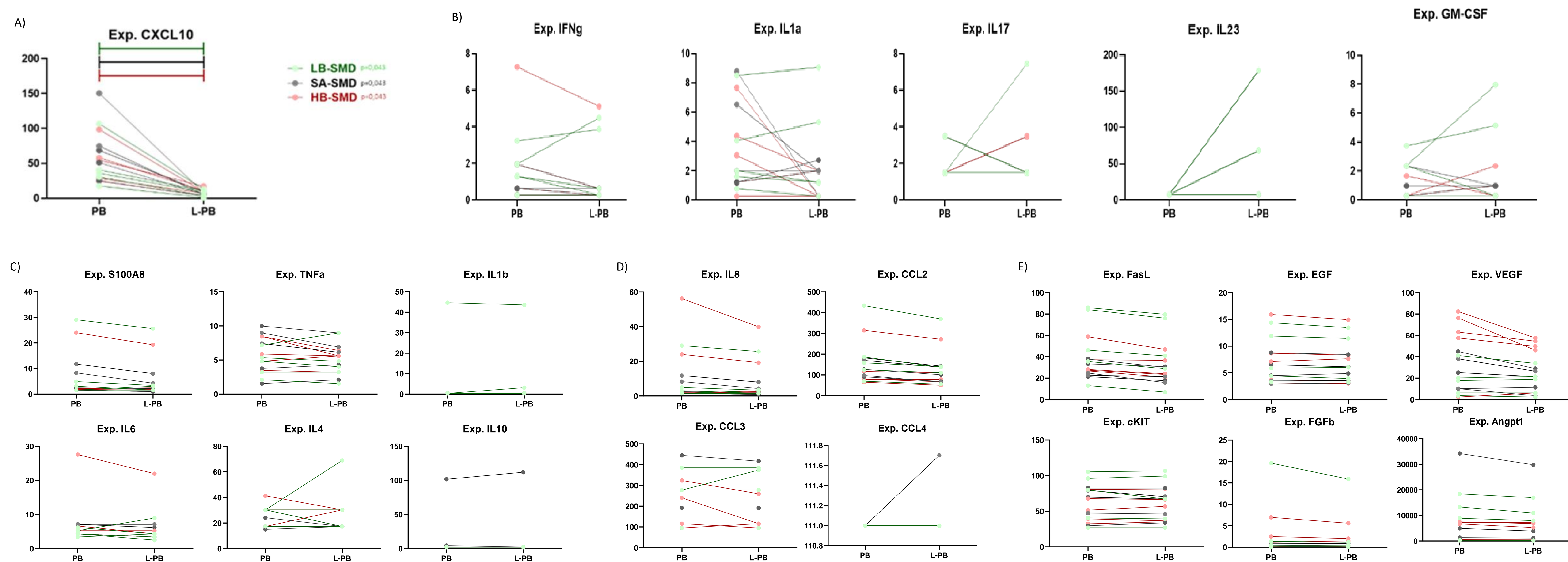

Figure 2: A) CXCL10 concentration; B) Different cytokine concentration between frozen vs freeze-dried plasma C) Citokines involved in inflammation D) Chemokines E) Growth factors

CONCLUSION

The stabilization of plasma samples obtained from peripheral blood through the lyophilisation process here used, is a valid procedure for their preservation at room temperature, allowing their use for Luminex cytokine analysis. However, further studies will be necessary to determine their stability over time.

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